THE USE OF ASCORBIC ACID (CRYSTALLINE VITAMIN C) AS A SUBSTRATE IN OXIDASE MEASUREMENTS[1]

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INTRODUCTION

During storage the physiological changes in fruits are katabolic, and oxidative processes are doubtless dominant. A study of these processes is of importance from the standpoint of fruit behavior and particularly with reference to physiological diseases such as soft scald and break-down of apples and core break-down of pears. A few degrees difference in storage temperature may mean either severe losses or fruit comparatively free from disease. Thus, at 30° or 32° F. certain varieties of apples may develop severe soft scald, whereas at 36° this disease very seldom develops. However, at the higher temperature the period of profitable storage is shortened owing to more rapid ripening. These results indicated that a study of the oxidizing enzymes might be of considerable importance for an understanding of the basic causes of this type of disease and in turn might lead to methods for its control. Preliminary tests indicated that the difference in oxidase activity of fruit stored at 32° and 36° might be small. A more sensitive method than had previously been available for measuring small changes in enzyme activity was needed, and the present study was therefore undertaken.

Oxidase activity may be determined colorimetrically (*16, p. 234*),[2] volumetrically (*4*), or iodometrically (*3*). Of these three processes, the last is in many respects the most satisfactory. In Guthrie's (*3*) iodometric method the oxidizable substrate is prepared by heating glucose with a dilute sodium hydroxide solution. The glucose is broken down into numerous derivatives, some or all of which reduce iodine in acid solution. These carbohydrate degradation products are unstable and lose their oxidizing power in the presence of air, and their oxidation is catalyzed by plant juices, i. e., by oxidase.

When glucose is heated with dilute alkali, a mixture of various decomposition products is formed. It is conceivable that some of these carbohydrate derivatives may require different degrees of oxidation before their iodine-reducing power is destroyed, while other products in this mixture may possess no iodine-reducing power or may even have an inhibitory action on the oxidase enzyme. For this reason it would seem desirable to have a pure substrate in which the oxidizable material would be uniform and other products would not be present as a possible source of interference with the enzyme action.

In the quantitative determination of ascorbic acid in plant tissues it is necessary to inactivate the plant oxidase to prevent destruction of the acid. Szent-Györgyi (*13*) noted the reducing power of plant juices and identified the reducing substance as hexuronic acid. This

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[2] Italic numbers in parentheses refer to Literature Cited, p. 98.

was later termed ascorbic acid and is now available in crystalline form. Ascorbic acid, then, is a definite chemical compound; it may be obtained in pure form; it is readily oxidized by the oxidase enzyme; and the amount of oxidation may be determined by iodine reduction in acid solution. It therefore appeared likely, and preliminary tests indicated, not only that ascorbic acid could be used as a substrate for oxidase measurements but that it possessed distinct advantages over the glucose-derivative substrate as prepared by Guthrie.

This paper presents the results of a study in which ascorbic acid (crystalline vitamin C) is used as a substrate for oxidase measurements in comparison with the glucose-derivative substrate of Guthrie. The influence of concentration of the enzyme, of the ascorbic acid, of the acidity, and of various other factors has received attention.

MATERIALS AND METHODS

The enzyme preparation consisted of the pressed juice in most cases. The tissue to be used was ground in a food chopper with a nut-butter attachment, and the juice was squeezed through two thicknesses of cheesecloth. The juice was then filtered through double cheesecloth, without pressure, to remove any tissue forced through at the first filtering.

The method of measuring oxidase activity was similar to that described by Guthrie (*3*) except that 25 cc. of a water solution of ascorbic acid crystals (cevitamic acid, Merck) was used, usually in concentrations of 0.1 to 0.4 percent, as a substrate in the Van Slyke-Cullen aeration tubes, instead of the heated glucose solution of Guthrie. From 1 to 10 cc. of the juice or extract containing the enzyme was added to each tube, the amount depending on the oxidase content. The boiled preparation was used as a blank. Whenever the enzyme-substrate solution foamed badly, 5 drops of paraffin oil were added to each tube. Uniform reaction conditions were obtained by drawing air for 1 hour, at the rate of 8 liters per hour, through a constant-temperature bath held at 25° C. After aeration the solutions were transferred with 50 cc. of water to 500-cc. Erlenmeyer flasks containing 25 cc. of 10-percent trichloroacetic acid. Twenty cubic centimeters or more of N/50 iodine in N/10 potassium iodide was then added, and after standing for 30 minutes the solution was titrated with N/100 sodium thiosulfate. The difference in titration between the boiled and the unboiled sample gave a measure of the oxidase activity. When several samples are aerated simultaneously, care should be taken to keep the iodine oxidation-reduction period uniform.

EXPERIMENTAL WORK

EFFECT OF THE HYDROGEN-ION CONCENTRATION OF THE SUBSTRATE ON OXIDASE ACTIVITY

Water solutions of ascorbic acid are acid in reaction. Falk (*2*, *p. 98*) states that the optimum pH value for vegetable oxidase activity is from 7 to 10. This is more alkaline than most plant juices. Apple juice has a pH value of approximately 4. With potato juice and a sugar-derivative substrate, Guthrie (*3*) suggests a pH value of 6.5 for oxidase measurement.

In order to determine whether the acidity of the ascorbic acid solution interfered seriously with the oxidase activity, this substrate

was adjusted to various acidities with N/10 sodium hydroxide and buffered with Clark and Lubs buffer solutions. Equal parts of the substrate and of buffer solutions were used and adjusted to a constant volume and concentration. The oxidase activity of apple juice was then determined at various acidities. The results are given in table 1, column 2.

The substrate with a pH value of 4.0 was more acid than is usually recommended for optimum oxidase activity, but the use of sodium hydroxide and buffer solutions to give a pH value as high as 8.0 did not increase the enzyme activity. The fact that there was such a wide difference between the check (unbuffered substrate) and the sample buffered to pH 4.0, both of which were near the same acidity, suggested that the buffer chemicals were interfering with the enzyme action. This proved to be true, as is shown in table 1, column 3, when the substrate was brought to the desired pH value with N/10 sodium hydroxide without addition of buffer. With the acidity thus reduced enzyme activity was again less than in the unneutralized substrate.

TABLE 1.—*Effect of the hydrogen-ion concentration of the substrate on oxidase activity*

Ascorbic acid substrate (pH) [1]	Oxidase activity (N/100 $Na_2S_2O_3$)		
	Apple juice (substrate buffered)	Apple juice (substrate unbuffered)	Potato juice (substrate unbuffered)
(1)	(2)	(3)	(4)
	Cc.	*Cc.*	*Cc.*
Check [2]	6.2	8.5	7.1
4.0	.7	8.5	7.1
5.0	2.6	6.6	6.5
6.0	3.8	6.1	7.3
7.0	2.7	5.2	7.1
8.0	1.5	5.2	7.2

[1] N/10 NaOH added to secure pH value desired.
[2] 0.1-percent water solution, pH value approximately 4.0.

Since apple juice has a pH value approximately equal to a 0.1-percent solution of ascorbic acid, it was thought that an enzyme preparation less acid than apple juice might behave differently in regard to the acidity of the substrate. Although repeated tests with potato juice having a pH value of 6.2 did not always show increased oxidase activity with the more alkaline substrates, the effect of adjustment did not appear to be harmful. The results of one of these tests are given in table 1, column 4. These results with apple and potato juice might indicate that the optimum pH value for oxidase activity is near that of the original material; i. e., apple near pH 4.0 and potato near pH 6.2. The failure of the potato juice to show definitely greater activity with the adjusted substrates may be due to the injurious effect of the sodium hydroxide on the enzyme, offsetting the advantages of increase in alkalinity.

EFFECT OF CONCENTRATION OF SUBSTRATE ON OXI DASE ACTIVITY

The effect of concentration of the substrate on the oxidase activity of apple juice is shown (table 2) under conditions in which the concentration of the ascorbic acid ranged from 0.1 to 0.4 percent. Two cubic centimeters of apple juice was used at each concentration.

Differences in concentration of the substrate from 0.1 to 0.3 percent of ascorbic acid had little effect on the enzyme activity, whereas 0.4 percent was strong enough to cause injury to the enzyme (table 2, column 2). Guthrie (*3*) has stated that boiled juice has a protective effect on the oxidase enzyme. When 8 cc. of boiled juice was added to 2 cc. of unboiled juice and the test repeated, no injury at 0.4 percent was evident (table 2, column 3). In a later test, with onion juice very weak in oxidase, a 0.3-percent ascorbic acid solution showed slight injury to the enzyme.

TABLE 2.—*Effect of concentration of substrate on the oxidase activity*

Concentration of substrate (percent)	Oxidase activity (N/100 $Na_2S_2O_3$)	
	Enzyme unprotected	Enzyme protected [1]
(1)	(2)	(3)
	Cc.	*Cc.*
0.1	9.9	12.8
0.2	9.9	13.0
0.3	9.9	13.0
0.4	8.0	13.0

[1] Enzyme protected by the addition of 8 cc. of boiled juice to 2 cc. of unboiled juice.

For routine analysis, where the titration difference between the boiled and the unboiled juice is not more than 15 cc. of N/100 thiosulfate, a substrate concentration of 0.1 percent of ascorbic acid has been found satisfactory. Since 1 cc. of N/100 thiosulfate is equivalent to 0.88 mg. of ascorbic acid (*8*), a titration of 15 cc. would indicate that 13.20 mg. of ascorbic acid had been oxidized by the enzyme. The amount oxidized by the blank varies with the product and with the amount of boiled material used: 2 cc. of boiled apple juice oxidized 1.85 mg. of ascorbic acid during aeration; a difference of 15 cc. in titration would indicate that approximately 60 percent of the substrate had been oxidized.

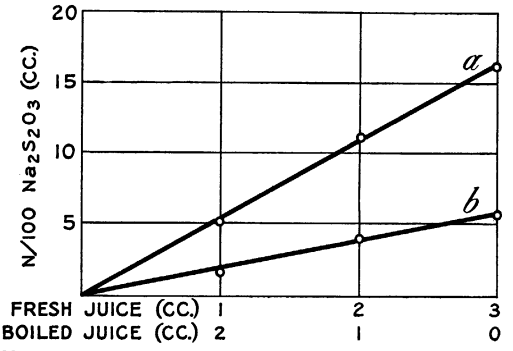

FIGURE 1.—Effect of concentration of the enzyme on the oxidase activity of apple juice: *a*, Relatively strong oxidase solution; *b*, relatively weak oxidase solution

EFFECT OF CONCENTRATION OF ENZYME ON OXIDASE ACTIVITY

When the protective action of the juice is kept constant by the addition of boiled juice, the oxidase activity varies directly with the concentration of the enzyme. This is shown in figure 1, where the activity of apple juice relatively strong and weak in oxidase is plotted.

EFFECT OF THE SUBSTRATE AND ITS OXIDATION PRODUCTS ON THE ENZYME

The product of a reaction often interferes with the action of the enzyme, and as a result the concentration of active enzyme may change during the course of the reaction. For instance, when 2-cc. samples of Winesap apple juice were aerated for 20, 40, and 60 minutes, the oxidase activity was 3.4, 6.7, and 9.7 cc., or 3.4, 3.3, and 3.0 cc. for the three 20-minute intervals, respectively. This reduction in enzyme activity may be due to the action of the substrate or to the products formed by the reaction between the enzyme and substrate. Guthrie (*3*) noted that this destructive action was reduced by the addition of boiled juice to the unboiled juice. To determine whether the ascorbic acid substrate was as injurious to the enzyme as the glucose-derivative substrate, a test was made in which both ascorbic acid and glucose-derivative substrate were used. In each case the quantity of unboiled juice was kept constant while the quantity of boiled juice was varied. The results are shown in table 3.

It is evident that the boiled juice was more effective in increasing oxidase activity with the heated glucose-derivative substrate than with the ascorbic acid substrate. Also the difference between 2 and 4 cc. of boiled juice was much greater in the glucose-derivative substrate than in the ascorbic acid substrate. Conversely, it may be stated that the heated glucose-derivative substrate or the products of its oxidation are more destructive to the enzyme than is the ascorbic acid substrate or its oxidation products. The substrate, reduced or oxidized, should be as free as possible from such destructive substances.

TABLE 3.—*Effect of different substrates on the oxidase activity of apple juice when various proportions of protective boiled juice are used*

Enzyme preparation	Activity of apple juice on—			
	Ascorbic acid substrate		Glucose-derivative substrate	
	Oxidase activity (N/100 $Na_2S_2O_3$)	Increase over unprotected juice	Oxidase activity (N/100 $Na_2S_2O_3$)	Increase over unprotected juice
	Cc.	*Percent*	*Cc.*	*Percent*
2 cc. of unboiled juice, no boiled juice	7.0		4.2	
2 cc. of unboiled juice + 2 cc. of boiled juice	9.4	34.3	6.3	50.0
2 cc. of unboiled juice + 4 cc. of boiled juice	10.0	42.8	7.8	85.7

Johnson and Zilva (*5*) have shown that certain plant enzymes oxidize catechol in catechol-ascorbic acid mixtures but only after the ascorbic acid is all oxidized. This would indicate that ascorbic acid is more readily oxidized than catechol. If increasing amounts of an enzyme preparation are added to a constant amount of ascorbic acid substrate until an excess of enzyme is present, a slight excess of enzyme shows no increase in oxidase activity and no difference in the color of the solution. However, if an additional excess is added, further visible oxidation takes place and darkening results, the amount of darkening depending roughly on the excess enzyme added. It should be noted that visible darkening does not take place until all of the ascorbic acid is oxidized. According to Onslow (*10, p. 122*) oxidase

is made up of three components, one of which is an aromatic substance containing an orthodihydroxy grouping such as that in catechol. The darkening may be due to the oxidation of this aromatic compound. This would seem to agree with Johnson and Zilva's results, in which the catechol, in ascorbic acid-catechol mixtures, was not oxidized until all of the ascorbic acid had been oxidized.

The direction of the ascorbic acid oxidation appears somewhat obscure. Ascorbic acid is generally considered to be first oxidized to dehydroascorbic acid, which is approximately equal to ascorbic acid in antiscorbutic potency. According to Borsook et al. (*1*), dehydroascorbic acid in aqueous solutions undergoes a spontaneous irreversible change at ordinary temperatures if the hydrogen-ion concentration is less than pH 4, forming a stronger acid and a more powerful reducing agent than ascorbic acid itself. However, this change is independent of the presence of air or oxidizing agents; consequently it is not an oxidation. Moll and Wieters (*9*) found that pure aqueous solutions of dehydroascorbic acid are far less stable than ascorbic acid solutions, and that the antiscorbutic property is lost more quickly than the property of being regenerated by hydrogen sulfide. This degradation product is an ascorbic-acid-like substance with a characteristic reductive action on iodine, silver nitrate, and dichlorphenolindophenol, but without antiscorbutic properties. In order to determine whether the products of the ascorbic acid oxidation were reducing the iodine in a similar manner to the unoxidized ascorbic acid the following study was made, in which the enzyme was permitted to act on the substrate over a long period of time and in which the oxidation products were allowed to stand in strongly acidified solutions. The results are as follows:

Lot No. and treatment:	Oxidase activity (cc. N/100 $Na_2S_2O_3$)
(1) Check, 2 cc. of enzyme material, aerated 1 hour; oxidase determined by usual procedure	2. 9
(2) 2 cc. of enzyme material, aerated 1 hour and then let stand for 17 hours at 27° C. before adding acid and iodine and determining the activity	4. 6
(3) 2 cc. of enzyme material, aerated 1 hour; then 25 cc. of 10-percent trichloroacetic acid added and let stand 17 hours at 27° C. before iodine added and activity determined	3. 0
(4) 10 cc. of enzyme material, aerated 1 hour, but let stand 17 hours at 27° C. before acid and iodine added and activity determined	[1] 23. 6
(5) 10 cc. of enzyme material, aerated 1 hour; then 25 cc. of 10-percent trichloroacetic acid added and let stand 17 hours at 27° C. before iodine added and activity determined	[1] 20. 0

[1] All of the substrate was oxidized in lots 4 and 5. The apparent difference in activity was due to difference in the blanks. The blank in lot 5, acidified following aeration, was greater than the blank in lot 4. The thiosulfate titration was practically the same in all tubes containing the enzyme in the 2 lots.

From the above data, it appears that the degradation products did not react with the iodine as the ascorbic acid did even when the enzyme was allowed to act for 18 hours or when the oxidized substrate was allowed to stand in a strongly acidified solution for 17 hours. Lot 1 was a comparatively weak enzyme solution and oxidized but little of the substrate when determinations were made by the usual procedure. Lot 2, in which the enzyme was allowed to act without aeration for an additional 17 hours, showed 58 percent more of the substrate oxidized than lot 1. Lot 3 had the enzyme killed at the end of aeration and, as would be expected if the oxidized substrate

did not react with the iodine, showed practically the same activity as lot 1. Lots 4 and 5, containing 10 cc. of the enzyme, oxidized all the substrate in less than 1 hour as was shown by a brownish discoloration at the end of the aeration. Lot 4 showed increased darkening after 18 hours and was distinctly darker at the end of the period than lot 5, in which the enzyme was killed at the end of aeration. Yet no lot gave any indication that the oxidized substrate was reducing the iodine as occurs in the case of ascorbic acid. The apparent difference in activity between lots 4 and 5 is due to a difference in the blanks; the acidified blank gave a higher thiosulfate titration than did the one that remained unacidified overnight. It should be recalled that the thiosulfate titration is a measure of the excess iodine added after the enzymic oxidation of the ascorbic acid; it is directly proportional to the enzyme activity and inversely proportional to the ascorbic acid remaining in the substrate.

Even though the reducing substance formed from dehydroascorbic acid is present in the oxidized substrate, it still would be ineffective in reducing the iodine. Borsook et al. (*1*) have shown that it is inactive as a reducing agent below pH 4. The acidified substrate, containing 25 cc. of 10-percent trichloroacetic acid to which the iodine is added, is considerably lower than pH 4. At no time has there been any indication of a reducing substance being formed in the oxidized substrate.

ENZYMIC VERSUS METALLIC OXIDATION OF THE ASCORBIC ACID

It is well known that copper can catalyze the oxidation of ascorbic acid. Stotz et al. (*12*) found that the copper in the so-called "ascorbic acid oxidase" from squash and cauliflower was sufficient to account for the oxidation credited to the enzyme and that the greater part of the copper could be recovered in the coagulated protein upon heat inactivation. In the present work practically the same results were obtained whether the enzyme was killed by heating or by the addition of 10-percent crystalline trichloroacetic acid to the juice. Other metals also may catalyze the oxidation of ascorbic acid (*7*). That grinding the tissue through the food chopper had no effect on the rate of oxidation of the ascorbic acid was shown by using ripe peaches in which the juice could be hand-pressed without cutting or grinding. Hand-pressed juice gave the same activity as did the juice from similar tissue ground in the food chopper before pressing.

That the oxidation of the ascorbic acid was due to an oxidizing enzyme and not to copper or other metals present in the juice is shown by the results of an experiment (table 4) in which the oxidase activity of the same enzyme preparations was determined at various intervals of time. The enzyme solutions were held in stoppered Erlenmeyer flasks at room temperature throughout the test. If the oxidation were due to copper, then with the copper content of the juice remaining constant the rate of oxidation of the substrate should also remain constant; but if the oxidation were due to an enzyme, the enzyme gradually would be destroyed with standing, and the rate of oxidation should decrease. The data in table 4 show that the ability to oxidize the substrate diminishes with time. This would indicate that the oxidation is enzymic in character.

Other indications that the oxidation is due to an enzyme and not to copper that might be present in the juice are given in table 3 and

on page 93. The addition of boiled juice (table 3) would not be expected to exert a protective action on the copper but might be expected to do so with enzymes. Also, the copper content being constant, the amount of oxidation would be expected to remain constant during equal intervals of time. That such is not the case is shown on page 93.

COMPARATIVE EFFECT OF DIFFERENT SUBSTRATES ON THE OXIDASE ACTIVITY OF VARIOUS PLANT PRODUCTS

The relative oxidase activity of various plant products was determined with ascorbic acid substrate and with the glucose-derivative substrate prepared as recommended by Guthrie (*3*). The results are given in table 5.

TABLE 4.—*Effect of time on oxidase activity*

Time elapsed after preparation	Oxidase activity (N/100 $Na_2S_2O_3$) of—	
	Delicious apple juice	Golden Delicious apple juice
	Cc.	*Cc.*
2 hours	9.5	8.0
26 hours	5.7	6.2
45 hours	2.1	3.8
15 days [1]	0	.5

[1] Slight growth of mold in solutions at this time.

TABLE 5.—*Oxidase activity of various plant products as influenced by the kind of substrate*

Source of enzyme		Oxidase activity (N/100$Na_2S_2O_3$)		Source of enzyme		Oxidase activity (N/100 $Na_2S_2O_3$	
Material	Quantity	Ascorbic acid substrate	Glucose-derivative substrate	Material	Quantity	Ascorbic acid substrate	Glucose-derivative substrate
	Cc.	*Cc.*	*Cc.*		*Cc.*	*Cc.*	*Cc.*
Winesap apple juice (fruit)	2	7.1	3.9	Cantaloupe juice (melon)	10	0.7	0.0
Red Rome apple juice (fruit)	5	8.1	5.5	Lettuce juice (head)	2	6.2	2.0
Bosc pear juice (fruit)	2	2.3	1.0	Cucumber juice (fruit)	2	26.1	15.5
Bartlett pear juice (fruit)	2	4.0	1.7	Watermelon juice (red meat)	10	0	0
Peach juice (fruit)	5	12.8	5.7	Orange juice (fruit)	10	0	0
Plum juice (fruit)	5	15.4	7.7				
Potato juice (tuber)	2	24.6	15.5	Average		6.52	3.28
Onion juice (bulb)	5	1.4	1.1				
Carrot juice (root)	5	8.3	1.3	Average increase over glucose-derivative substrate		*Percent* 98.7	
Radish juice (root)	5	0	0				
Cabbage juice (head)	2	1.6	.8				
Water suspension of—							
Dried cherry bark	2	1.2	0				
Do	4	2.0	.4				
Fresh cherry bark	5	2.4	.3				

It is interesting to note that the average oxidase activity of these various plant products was 98.7 percent greater with the ascorbic acid substrate than with the sugar-derivative substrate. This greater activity is especially important where the amount of enzyme activity is small. In some cases where no activity was evident with the glu-

cose-derivative substrate, definite activity was shown with the ascorbic acid substrate. That the ratio of activity for the substrates did not remain constant with different enzyme preparations was probably due to the varying amounts of the injurious substances in the sugar-derivative substrate or to the relative immunity of the various preparations to injury from such substances. Table 3 shows that the sugar-derivative substrate was more injurious to the enzyme than was the ascorbic acid substrate.

DISCUSSION

In the determination of ascorbic acid in plant tissues by dichlorphenolindophenol or other chemical means it is often necessary to inactivate the plant oxidase to prevent the ascorbic acid from being oxidized to dehydroascorbic acid, which is biologically active but does not react with the dichlorphenolindophenol dye. Plants differ considerably in the rate at which this change takes place. Stone (*11*) concluded that it was a question of ascorbic acid oxidase. If the oxidase was present the plant lost the indophenol-reducing power on mincing; if the oxidase was absent the indophenol-reducing power remained.

Several workers (*5*, *6*, *11*, *13*, *14*, *15*) have reported the presence of an ascorbic acid oxidase that can oxidize ascorbic acid. Szent-Györgyi (*13*), the first to note this property, states that the hexuronic acid (ascorbic acid) oxidase is not a single enzyme but apparently involves a number of catalysts. Tauber et al. (*15*) isolated an ascorbic acid enzyme from the pericarp of Hubbard squash which gave none of the color reactions that other oxidases give with such reagents as benzidine, guaiacol, pyrogallol, catechol, phloroglucinol, resorcinol, naphthoresorcinol, or vanillin. Nor did it affect glutathione, cysteine, tyrosine, adrenalin, or glucose boiled with alkali, and its kinetics indicated a single enzyme. However, they found practically no ascorbic acid, reduced or oxidized, in the fruit, so the role of this enzyme in the physiology of the plant remained obscure.

Copper catalyzes the oxidation of ascorbic acid very readily, and precautions must be taken to prevent it from oxidizing the ascorbic acid in the dichlorphenolindophenol method of analysis (*7*). Stotz et al. (*12*) studied the role of copper in the ascorbic acid oxidase from squash and cauliflower and reported that the copper content was sufficient to account for the observed catalysis and that the greater part of the copper in such preparations was found in the coagulated protein upon heat inactivation. Additional results strengthened the indication that copper was responsible for the oxidation of the ascorbic acid. Stotz et al. (*12*) denied any need for using such terms as "ascorbic acid oxidase," "vitamin C oxidase," or "hexuronic acid oxidase," at least in the sense that they have been used in the past.

However, it is generally recognized, and the work reported herein emphasizes, that there are certain plant enzymes that are capable of oxidizing ascorbic acid. These same enzyme preparations oxidize the degradation products formed when glucose is heated with an alkali, and thus seem to be the same as or closely associated with indophenol oxidase (*3*). Indirectly, these results would support Stotz et al. (*12*) in their belief that no special "ascorbic acid oxidase" is necessary to account for the oxidation of ascorbic acid.

SUMMARY

The use of ascorbic acid as a substrate in oxidase measurements is suggested. The effects of various factors, including the pH value of the substrate, concentration of the substrate, and concentration of the enzyme, were determined. Ascorbic acid was shown to be less injurious to the enzyme than a glucose-derivative substrate. In the case of numerous plant products, the oxidase activity with an ascorbic acid substrate averaged 98.7 percent greater than with a glucose-derivative substrate. This greater sensitivity should permit measurements of smaller changes in oxidase activity of fruits in storage. It should find application in investigations not only of soft scald but also of certain other physiological diseases wherein oxidative changes may be a causative factor. Data are included to show that the oxidation of the ascorbic acid is enzymic and not metallic.

Ascorbic acid substrate possesses the following advantages over a glucose-derivative substrate: (1) Ascorbic acid is a definite chemical compound available in pure form; (2) it is more readily oxidized, enabling the investigator to note smaller differences in enzyme activity; (3) it is water-clear in solution, facilitating titration; (4) it is less injurious to the enzyme; and (5) its use as a substrate saves time, since ascorbic acid is readily soluble in water and a fresh solution may be prepared in a few minutes.

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